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L3: Entry 2 of 6 File: USPT Nov 2, 1999

DOCUMENT-IDENTIFIER: US 5976845 A
TITLE: Composite antibodies of human subgroup IV light chain capable of binding to TAG-72

DEPR:
DNA encoding an antibody light chain was isolated from a sample of blood from a human following the protocol of Madisen et. al. (1987), Am. J. Med. Genet., 27:379-390), with several modifications. Two 5 mL purple-cap Vacutainer tubes (containing EDTA as an anticoagulant) were filled with blood and stored at ambient temperature for 2 hours. The samples were transferred to two 4.5 mL centrifuge tubes. To each tube was added 22.5 mL of filter-sterilized erythrocycte lysate buffer (0.155 M NH.sub.4 Cl and 0.17 M Tris, pH 7.65, in a volume ratio of 9:1), and incubated at 37.degree. C. for 6.5 minutes. The tubes became dark red due to the lysed red blood cells. The samples were centrifuged at 9.degree. C. for 10 minutes, using an SS-34 rotor and a Sorvall centrifuge at 5,300 revolutions per minute (rpm) (.sup..about. 3,400.times.g). The resulting white cell pellets were resuspended in 25 mL of 0.15 M NaCl solution. The white blood cells were then centrifuged as before. The pellets were resuspended in 500 .mu.L of 0.15 M NaCl and transferred to 1.5 mL microcentrifuge tubes. The cells were pelleted again for 3 minutes, this time in the microcentrifuge at 3,000 rpm. Very few red blood cells remained on the pellet. After the supernatants were decanted from the 2 microcentrifuge tubes, 0.6 mL high TE buffer (100 mM Tris, pH 8.0) was added. The tubes were hand-shaken for between 10 and 15 minutes. The resulting viscous solution was extracted with phenol, phenol-chloroform and finally with just chloroform as described in Sambrook et al., supra. To 3.9 mL of pooled extracted DNA solution were added 0.4 mL NaOAc (3 M, pH 5), and 10 mL 100 percent ethanol. A white stringy precipitate was recovered with a yellow pipette tip, transferred into a new Eppendorf tube, washed once with 70 percent ethanol, and finally washed with 100 percent ethanol. The DNA was dried in vacuo for 1 minute and dissolved in 0.75 mL deionized water. A 20 .mu.L aliquot was diluted to 1.0 mL and the OD 260 nm value was measured and recorded. The concentration of DNA in the original solution was calculated to be 0.30 mg/mL.

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L2: Entry 3 of 27 File: USPT Feb 1, 2000

DOCUMENT-IDENTIFIER: US 6020153 A
TITLE: Chimeric antibodies

DEPR:
The solution is extracted by mixing with an equal volume of redistilled phenol saturated with 20 mM Tris-HCl, pH 8.0, at 0.degree. C. The aqueous phase is recovered after centrifugation (10,000 rpm, room temperature, Sorvall RC-5 Centrifuge, SS-34 Rotor) and extracted twice with an equal volume of CHCl.sub.3 /isoamyl alcohol (24:1, v/v). DNA is precipitated by the addition of one-tenth volume of 3M NaOAc, pH 5.0, followed by two volumes of absolute ethanol at room temperature. The precipitated DNA is lifted from the ethanolic solution, placed in 1 ml of TE buffer and dissolved overnight at 4.degree. C. The yield of DNA is approximately 0.5 mg.

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L2: Entry 4 of 27 File: USPT Jan 11, 2000

DOCUMENT-IDENTIFIER: US 6013435 A
TITLE: Drug resistance screening method using multiplex amplification

BSPR:
The nucleic acid amplification reactions are preferably performed using clinical specimens suspected of containing infectious agents, however, it will be apparent to those skilled in the art that the inventive methods are applicable to testing for drug resistance developed by any type of cell. The specimens can be blood samples, tissue samples, urine, sputum, throat swabs, exudates or other clinical specimens normally tested in the diagnosis of infection. Although it is not always required, it is preferable to at least partially purify nucleic acid from the specimen prior to amplification in order to obtain optimal efficiency of the amplification reactions. For example, after disruption of cells in the specimen, nucleic acid can be extracted from contaminating cell debris and other protein substances by extraction of the sample with phenol. In phenol extraction, the aqueous sample is mixed with an approximately equal volume of redistilled phenol and centrifuged to separate the two phases. The aqueous phase, containing the nucleic acid, is removed and precipitated with ethanol to yield nucleic acid free of phenol for use in the amplification reactions. Alternatively, target DNA can be purified according to the method of Vogelstein and Gillespie (PNAS 76:615, 1979).

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